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**Metalloprotein Composition Determination Using Capillary Electrophoresis With X-ray Fluorescence Spectroscopy**

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Capillary electrophoresis (CE) has been combined with X-ray fluorescence (XRF) to create a novel technique for the detection of metals in metalloproteins. CE has been used for many years for the separation of proteins, based on the mobilities of ions in an electric field. UV detection is the most commonly used method of detection, with mass spectrometry also being used for protein analysis. Although these methods are very informative, they do not give direct information about the metals bound to the protein.

XRF is intrinsically metal specific. Every element has a unique excitation and emission energy, so two metals can always be distinguished from one another. However, fused silica capillary is not transparent to x-rays at high energies. Therefore, a coupling made of polyethylene was created to enable XRF detection during a CE separation. Figure 1 is a schematic of the capillary electrophoresis with x-ray fluorescence (CE-XRF) system.

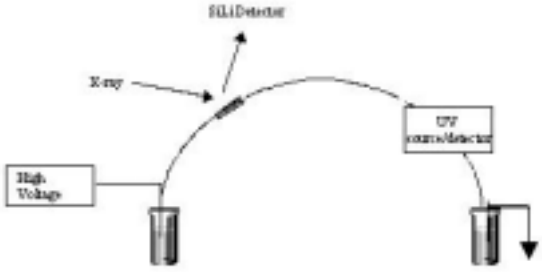


**Figure 1**. Schematic of CE – XRF system.

Although 30% of all proteins are thought to be metal-binding proteins, very little is known about the metal content of these proteins, and even less is known about the *in vivo* metal loadings of proteins. The study of metals in biological systems presents many challenges. A widely used approach is to use gel electrophoresis to separate protein, and autoradiography to identify radioactive isotopes of metals bound to the proteins. However, this technique is limited to metals for which sensitive isotopes are available, cannot be used with some proteins in which the metals are weakly bound, and can present experimental difficulties due to the requirements for radioactive sample handling.

In the past year, we have used CE-XRF to look at a mixture of metalloproteins in solution. A mixture containing hemoglobin (Hb), carbonic anhydrase I (CAI), carbonic anhydrase II (CAII), and superoxide dismutase (SOD) was separated and detected using CE-XRF. These metalloproteins contain iron (Hb), zinc(CAI, CAII, SOD) and copper (SOD). UV detection was also performed on each separation to analyze the protein content. As can be seen in figure 2, four peaks are present for the four proteins, with the zinc and copper for SOD. The UV electropherogram also shows four peaks, corresponding to CAI, CAII, SOD and Hb (from left to right).

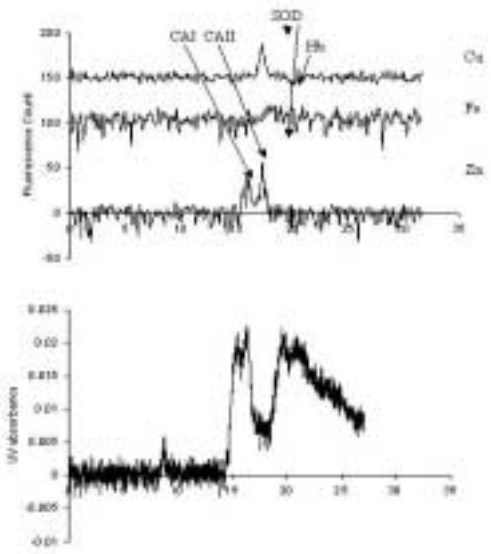


**Figure 2.** XRF electropherogram and UV electropherogram from the separation of Hb, CAI, CAII and SOD. Concentration of each protein was 5 mg/mL in 50 mM sodium borate solution, pH 9.5. Injection was by siphoning at a height of ½" for 30 seconds.

Metal concentrations can be obtained from the XRF data. These, when combined with protein concentrations from the UV data, can be used to determine the metal to protein ratios. The results from these calculations are given in Table 1. As can be seen, the calculated values were within 15% of the expected values.

| | CAI (Zn) | CAII (Zn) | SOD (Zn) | SOD (Cu) | Hb (Fe) |
|---|---|---|---|---|---|
| [illegible] | 2.11 | 1.89 | 4.46 | 5.10 | 7.57 |
| [illegible] | 2.10 | 2.15 | 4.46 | 4.46 | 2.03 |
| [illegible] | 0.99 | 0.87 | 0.98 | 1.13 | 3.49 |
| [illegible] | 1 | 1 | 1 | 1 | 4 |

**Table 1.** Calculated and expected metal to protein ratios. It can also be seen that within the Cu/Zn SOD, the Cu to Zn ratio is ≈ 1 : 1.

The combination of CE with XRF has been shown to be a valid method for the detection and quantification of metalloproteins. When combined with UV absorbance, metal to protein ratios can be determined in a single separation, in an on-line, non-destructive manner. Currently, proteins of unknown composition are being studied using the CE-XRF system.